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Antibody Response to Human Cytomegalovirus (HCMV) Glycoprotein B (gB) in AIDS Patients With HCMV End-Organ Disease

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Human cytomegalovirus (HCMV)-specific antibody responses in HIV-1 infected individuals either with or without HCMV end-organ disease were examined to determine the whether development of HCMV disease was associated with a particular deficit in the antibody response. Anti-whole HCMV, anti-glycoprotein B (gB), and neutralizing antibody levels were higher in HIV-1 infected individuals than in healthy immunocompetent subjects, particularly in patients with AIDS either with or without HCMV-associated disease. Irrespective of location and spread of HCMV disease, patients who had received anti-HCMV therapy prior to sampling exhibited significantly higher anti-gB and neutralizing antibody titers than those who remained untreated. Likewise, patients with HCMV disease who were antigenemic or viremic had significantly lower anti-gB and neutralizing antibody titers than those who tested negative in either assay. Patients with untreated HCMV disease had significantly lower antibody titers than AIDS patients without disease. Analysis of the IgG subclass antibody responses to gB revealed no significant differences among HIV-1 infected individuals. These results suggest that levels of detectable anti-gB and HCMV neutralizing antibodies are inversely related to systemic viral load. Thus, antibodies with such specificities may be relevant in preventing the establishment of HCMV-associated disease or in modulating its progression ***J. Med. Virol. 55:272–280, 1998.***

KEY WORDS: antibodies to gB; neutralizing antibodies; HCMV end-organ disease; AIDS patients

INTRODUCTION

Human cytomegalovirus (HCMV) end-organ disease is a serious clinical problem in patients with AIDS. Data reported prior to the implementation of highly active anti-retroviral molecules in the treatment of HIV infection reveal that up to 40% of patients with AIDS and CD4+ cell counts below 50 cells/mm^3 eventually develop HCMV symptomatic disease, most frequently in the form of retinitis or gastrointestinal disorders [Drew, 1992; Gallant et al., 1992]. A larger proportion of these individuals in turn present evidence of active HCMV replication in autopsy tissues [Reichert et al., 1983]. However, it is not known why a subset of patients with advanced AIDS remain free of symptomatic HCMV disease despite being severely immunosuppressed. Although cellular immunity appears to be crucial for the control of HCMV infection, several studies conducted in humans [Boppana and Britt, 1995; Boppana et al., 1993; Snydman, 1990] and in mice [Joncic et al., 1994], suggest that HCMV-specific antibodies may play a role in the immune control of viral dissemination, and thus in the prevention of severe disease. The verification of a deficient antibody response to HCMV in patients who develop HCMV end-organ disease would support this assumption.

HCMV glycoprotein B (gB) is a major component of the viral envelope that plays a central role in viral entry and in virus spread from cell-to-cell [Navarro et al., 1993]. In addition, gB is highly immunogenic in humans [Cremer et al., 1985; Gonczol et al., 1986, 1990; Kniess et al., 1991; Navarro et al., 1997; Pereira et al.,

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1991] and elicits a dominant antiviral neutralizing response [Britt et al., 1988, 1990; Marshall et al., 1992, 1994; Rasmussen et al., 1991]. Consequently, specific anti-gB antibodies are likely to be involved in protection against HCMV.

The main objective of the present study was to determine whether the occurrence of HCMV end-organ disease was associated with a specific deficiency in the antibody response to HCMV gB. To this effect we quantitated serum levels of gB-specific antibodies in AIDS patients suffering from distinct HCMV diseases and compared them to those of AIDS patients who remained free of HCMV-associated disease. In addition to quantitating anti-gB antibodies, we measured the capacity of patient sera to neutralize free virus given the relevance of such an immunological mechanism in the process of viral clearance.

MATERIALS AND METHODS

Subjects and Samples

Serum and anticoagulated blood samples were obtained at a single time point from three population groups: 1) 28 asymptomatic HIV-1 infected individuals, 12 belonging to group A1 (CD4+ > 499/mm^3) and 16 to group A2 (CD4+ between 200–499/mm^3) according to the 1993 Surveillance case definition and staging system of Centers for Disease Control (CDC, Atlanta, GA). There were 13 females and 15 males. Mean patient age in this group was 27.3 years (range, 17–40). The risk factor was parenteral drug abuse in 26 individuals and unsafe heterosexual contacts in the remaining 2. None of these patients was under anti-retroviral therapy at the time of sampling. 2) 25 AIDS patients, 23 of whom belonged to CDC group C3 (CD4+ < 200/mm^3), and the remaining 2 to group C2 (CD4+ between 200–499/mm^3). Eighteen were males and seven females. Mean age in this group was 33.1 years (range, 21B49). The risk factor was parenteral drug abuse in 22 individuals, unsafe heterosexual contacts in 2 patients, and unsafe homosexual contacts in 1 subject. All these patients were attended at the San Francisco de Borja Hospital and were under Zidovudine therapy at the time of sampling. 3) 25 AIDS patients with confirmed HCMV end-organ disease. The mean age of these individuals was 33.6 years (range, 25–47). Eighteen were males and 7 females. The risk factor was parenteral drug abuse in 16 individuals, unsafe heterosexual contacts in 6 subjects, and homosexuality in the remaining 3. Thirteen patients had retinitis alone, 3 retinitis and colitis, 2 retinitis and encephalitis, 1 retinitis and polyneuropathy, 3 colitis, 1 pneumonitis, 1 polyneuropathy, and 1 retinitis, adrenalitis, and colitis, concomitantly. In addition, sera from 19 immunocompetent individuals (10 males and nine females, mean age: 28.7 years; range, 19–40) were included as controls. Five additional sera obtained from HCMV-seronegative subjects were used as negative control sera in immunoassays. Formal consent was obtained from individuals enrolled in the study. Sera were heat-inactivated at 56°C for 30 min before use. Anticoagulated blood samples were processed as described below.

Diagnostic Criteria

The diagnosis of HCMV retinitis was based on the observation of characteristic retinal changes (perivascular exudates and hemorrhages) by ophthalmoscopic examination. Gastrointestinal disease due to HCMV was diagnosed by demonstrating typical CMV inclusions and expression of HCMV-early antigen by immunohistochemical procedures in biopsy specimens, in the absence of other common pathogens capable of causing invasive gastrointestinal disease. The diagnosis of encephalitis in patients with central neurologic symptoms was based on the observation of typical inclusions in brain tissue obtained at autopsy and by detecting HCMV DNA in CSF by PCR. Adrenalitis due to HCMV was diagnosed by the observation of typical CPE in autopsy specimens. HCMV-related polyneuropathy was diagnosed on the basis of a typical clinical syndrome along with a positive HCMV DNA PCR in CSF. Finally the diagnosis of HCMV pneumonitis was established by the isolation of HCMV in branchoalveolar lavage (BAL), the presence of typical cytopathic effects in biopsy specimens and the absence of other respiratory pathogens.

Virus and Cells

The HCMV AD169 laboratory strain (obtained from the ATCC collection) was used for the experiments reported in this study. Virus was propagated in low passage human foreskin fibroblasts (HFF) cells (ATCC) grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS) and antibiotics. A glioblastoma cell line expressing full length gB (U373-gB) was kindly provided by Prof. L. Pereira (UCSF) and used for immunofluorescence assays. Procedures for construction of the cell line as well as the characterization of its phenotypic properties were published previously [Tugizov et al., 1994]. Expression of HCMV gB was monitored periodically by indirect immunofluorescence using a gB-specific monoclonal antibody (CH177) [Qadri et al., 1992]. U373-gB cell line was maintained in DME medium containing 400 μg/ml of G418 (Genetycin, Sigma, St. Louis, MO). The parental glioblastoma cell line U373GM (ATCC) was propagated in DME medium and used as a control for the immunofluorescence assays.

CD4+ Quantitation

Quantitation of CD4+ lymphocytes was carried out by flow cytometry using the fluorescein-conjugated monoclonal antibody Leu-3a (Becton-Dickinson, Sunnyvale, CA) on a FACS-II analyzer (Becton-Dickinson).

Quantitation of Immunoglobulins

Total serum IgG was determined turbidometrically by a commercial nephelometric assay (Behring Diagnostics, Germany). Concentrations between 800 and

1800 mg/dl were considered to be within the normal range.

CMV Conventional Serological Assay

Total IgG antibodies to HCMV were measured by a commercial microparticle immunoassay (MEIA-IMx, Abbott Diagnostics, Germany) following the instructions of the manufacturer. Levels of anti-HCMV IgGs are given in AU (antibody units/ml). Sera giving AUs greater than 250 were not further quantitated and that value was considered for data analysis.

Viremia and Antigenemia Assays

For the detection of infectious virus in blood (viremia), buffy coats from heparinized blood were obtained by dextran sedimentation. Cells were washed twice in supplemented DME medium and a 0.5 ml fraction of each specimen was inoculated onto HFF cell monolayers grown on coverslips (shell vials). After 48 hours of incubation the coverslips were removed, fixed, and stained with an IE-1 specific murine monoclonal antibody (Chemicon International, Temecula, CA). Detection of pp65 in blood leukocytes (antigenemia) was carried out following a recent optimization of a standard procedure [Gerna et al., 1992]. Cells were stained with a pp65-specific monoclonal antibody obtained from Chemicon International. The result of this test was reported as being either positive or negative without quantitation.

HCMV gB Antibody Analysis

Anti-HCMV gB antibodies were measured by an immunofluorescence assay using the cell line U373-gB as the antigenic source, as previously described [Navarro et al., 1997; Pereira et al., 1993]. The cell line U3T3-gB expresses a full length gB molecule that retains the antigenicity of the native gB molecule as determined by extensive epitope analysis using a wide panel of well characterized murine moAbs [Tugizov et al., 1994]. Human sera were diluted serially in two-fold steps from 1/10 to 1/1,280 in phosphate-buffered saline (PBS) and incubated with gB-expressing cell (U3T3 gB) monolayers for 60 min at 37°C. Cells were washed three times with PBS and incubated with anti-human polyclonal IgG conjugated with FITC (Diagnostics Pasteur, Marnes-La Coquette, France; 1:1,000 in PBS) for 30 min. After washing, slides were mounted and observed under a Nikon epifluorescence microscope.

The gB titer was defined as the highest serum dilution that reacted specifically with gB-expressing cells. For gB-specific IgG subclass determinations, FITC-conjugated murine monoclonal antibodies to IgG1 (1:300), IgG2 (1:64), IgG3 (1:100), and IgG4 (1:64; Sigma Chemical Co.) were used. All immunofluorescence assays were performed in duplicate.

Virus Neutralization Assay

Neutralization titers of human sera were determined by a rapid neutralization assay basically following a published procedure [Navarro et al., 1993]. Briefly, human sera were diluted serially 1/10 to 1/1,280 in a 125 μl volume of DME medium and mixed with an equal volume of AD169 virus stock containing 50 plaque forming units. The mixtures were incubated at 37°C for 60 min and then plated onto HFF monolayers in 24-well plates.

After 60 min of incubation, mixtures were washed off and replaced by fresh DME medium. Forty-eight hours later, cells were fixed and stained with an IE-1 specific monoclonal antibody (Chemicon International) at 1:6,000 dilution in PBS. Fluorescent nuclei were counted and the neutralizing titer was defined as the highest serum dilution that reduced infectivity of the input virus by 50%. Neutralization assays were carried out in duplicate.

Data Analysis

Comparison of raw data between study groups was undertaken by the nonparametric Mann-Whitney U-test with the assistance of a commercially available software program (Instat, San Diego, CA). In this test, the average ranks of two independent samples (groups) are statistically compared. Two-tailed P values are given throughout the text. Two-tailed P values of <0.05 were considered to be of statistical significance. Correlations between raw values of different parameters were calculated using the Spearman rank correlation test.

RESULTS

Study Group Characteristics

A total of 78 HIV-1 infected subjects at different stages of the disease were enrolled in this study. Age and main HIV-acquisition risk factor (parenteral drug use) did not differ significantly among individuals. HIV-1 infected asymptomatic individuals had CD4+ cell counts of 216–842 cells/mm^3 with a mean count of 466.7 cells/mm^3. AIDS patients without HCMV disease had CD4+ cell counts of 6–98 cells/mm^3 = 20 (mean: 37.6 cells/mm^3), and AIDS patients with HCMV end-organ disease had CD4+ cell counts of 1–45 cells/mm^3 (mean: 19.6 cells/mm^3).

Table I shows some characteristics of the AIDS patients with HCMV end-organ disease included in this study. Of interest is the observation that 9 patients had received inductive or suppressive therapy with Ganciclovir, Foscarnet, or both within 1 month before serum sample collection. Of these patients, 7 had retinitis alone (3 primary episodes of symptomatic retinitis and 4 reactivations of previously inactive lesions), 1 retinitis and polyneuropathy, and 1 colitis. The remaining 16 individuals had not received specific anti-CMV therapy prior to sampling.

Qualitative HCMV viremia and antigenemia tests were performed in all HIV-1 infected persons. None of the HIV-1 asymptomatic individuals were viremic or antigenemic at the time of sampling. Five AIDS patients without HCMV-related disease were viremic, 3 of them having a positive antigenemia test. Among patients with HCMV end-organ disease, antigenemia was

TABLE I. Characteristics of AIDS Patients With HCMV End-Organ Disease

Patient no.	Age/sex	CD4+ (cell/mm^3)	HCMV disease	Antigenemia/ viremia	Previous treatment[a]
1	31/F	15	Retinitis	+/+	No
2	40/F	40	Retinitis	–/+	No
3	30/F	10	Retinitis	–/–	Yes
4	30/M	17	Retinitis	–/+	Yes
5	25/M	4	Retinitis	+/+	No
6	33/F	10	Retinitis	–/–	Yes
7	31/M	24	Retinitis	–/–	Yes
8	40/F	37	Retinitis	–/–	Yes
9	37/M	4	Retinitis	–/+	Yes
10	32/M	1	Retinitis	+/+	No
11	40/M	27	Retinitis	+/+	Yes
12	41/M	10	Retinitis	+/+	No
13	27/M	10	Retinitis	–/+	No
14	47/M	45	Retinitis Encephalitis	+/+	No
15	31/M	26	Retinitis Encephalitis	+/+	No
16	41/M	5	Retinitis Colitis	+/+	No
17	30/M	17	Retinitis Colitis	+/+	No
18	43/M	35	Retinitis Colitis	+/+	No
19	28/M	30	Retinitis Polyneuropathy	+/+	Yes
20	36/M	8	Colitis	+/+	No
21	30/M	12	Colitis	–/+	No
22	29/M	24	Colitis	–/–	Yes
23	31/M	8	Retinitis Colitis Adrenalitis	+/+	No
24	29/F	32	Polyneuropathy	+/+	No
25	29/F	14	Neumonitis	+/+	No

[a]Inductive or suppressive therapy with Ganciclovir, Foscarnet, or both within 1 month prior to sampling.

positive in 15 cases (13 untreated patients and 2 Ganciclovir-treated patients), while viremia was positive in 20 patients (16 untreated and 4 treated patients).

IgG Antibody Responses to Whole Virus

IgG antibodies to HCMV (whole virus) were measured by a standard commercial immunoassay. Patients with AIDS who developed HCMV end-organ disease had higher antibody titers than those who remained free of disease (Table II), although the difference was not statistically significant ($P = 0.509$; Table III). Overall, titers of HCMV-specific IgG antibodies were significantly higher in HIV-1 infected persons than in immunocompetent control individuals (see Table III for statistical significance of comparisons), and AIDS patients, particularly those with end-organ disease, had significantly higher antibody levels than HIV-1 asymptomatic individuals ($P = 0.0047$; Table III). The levels of HCMV-specific IgG antibodies did not correlate with individual CD4+ cell counts.

Quantitative Antibody Response to HCMV gB

To determine whether a quantitatively deficient antibody response to gB was related to the occurrence of HCMV end-organ disease, anti-gB antibodies were measured by an indirect immunofluorescence assay that uses a gB-expressing cell line (U3T3-gB) as antigen source. There was a weak correlation between the serum levels of IgG antibodies to whole virus and the individual anti-gB serum titers ($r = 0.3677$, 95% CI: 0.235–0.486, $P = <0.0001$). As shown in Table II, patients with AIDS and HCMV end-organ disease had gB antibody titers comparable to those of AIDS patients without HCMV-associated disease ($P = 0.08$, n.s.–Table III). Inasmuch as clinical profiles of patients with AIDS and HCMV end-organ disease appeared to be quite diverse (see Table I), for comparison purposes we subdivided further such group of patients into subgroups according to whether or not patients had received specific anti-HCMV therapy before serum samples were obtained and to the nature of the invasive disease present. The first subgroup included untreated patients ($n = 16$) with retinitis alone ($n = 6$), or with HCMV-invasive disease involving 2 or more anatomic sites or single organ involvement other than the retina ($n = 10$). A second subgroup was established in turn corresponding to treated patients ($n = 9$), 7 of whom had retinitis alone and 2 had a different clinical

TABLE II. Total IgG Levels and Anti-HCMV Antibody Titers in AIDS Patients With or Without HCMV End-Organ Disease

Study group	IgG level (mg/dl)	Antibody type (antibody titer) IgG anti-HCMV (whole virus)	IgG anti-gB	Neutralizing
AIDS with HCMV disease				
Mean	2,126.2	224.0	324.8	211.2
Median	1,978.0	250.0	160.0	80.0
Range	694–3,694	15–250	40–1,280	40–640
AIDS without HCMV disease				
Mean	2,284.7	201.2	332.8	291.2
Median	1,966.0	250.0	320.0	320.0
Range	946–5,238	18–250	80–1,280	40–640

TABLE III. Statistical Significance of Comparisons Between Study Groups

Study groups compared	Two-Tailed *P* Value[a] Total IgG	IgG anti-HCMV (whole virus)	IgG anti-gB	Neutralizing
AIDS with HCMV disease/AIDS without HCMV disease	0.7102	0.5096	0.0886	0.0287
AIDS with HCMV disease/HIV asymptomatic	0.2655	0.0047	0.5772	0.7526
AIDS with HCMV disease/Immunocompetent	0.0022	0.0005	0.0055	0.0053
AIDS without HCMV disease/HIV asymptomatic	0.2381	0.0934	0.0051	0.0039
AIDS without HCMV disease/immunocompetent	0.0038	0.0019	<0.0001	<0.0001
HIV asymptomatic/immunocompetent	0.0027	0.0770	0.0086	0.0089

[a]Statistical significance for *P* values <0.05.

condition (1 retinitis and polyneuropathy and 1 colitis). There were no statistically significant differences in the CD4+ cell counts between the two subgroups ($P = 0.514$). As shown in Table IV, patients who received specific anti-HCMV treatment had significantly higher gB antibody levels than untreated patients regardless of the concurrent HCMV end-organ disease ($P = 0.0064$). In this context, untreated patients with retinitis alone had gB antibody titers comparable to those found in untreated patients with HCMV invasive disease involving two or more anatomic sites or single organ involvement other than the retina ($P = 0.9578$; n.s). In addition, and irrespective of the HCMV disease present, untreated patients had lower gB antibody levels than AIDS patients without disease and matched CD4+ cell counts the difference being considered highly significant ($P = 0.0003$). In contrast, treated patients had more antibodies to gB than AIDS patients without disease, although the difference was not quite significant ($P = 0.06$). As shown in Table V, regardless of the HCMV-associated disease present, patients with antigenemia had significantly fewer anti-gB antibodies than those with a negative test ($P = 0.0054$). Likewise, patients who were viremic at the time of sampling had significantly lower anti-gB antibody levels than patients who were not viremic ($P = 0.0005$).

Differences in gB antibody titers between different groups or subgroups of HIV-1 infected individuals could not be related to the level of total IgG detectable in serum samples. As shown in Tables II and IV, total IgG levels as measured by nephelometry did not differ significantly between these groups. Furthermore, no correlation was found between individual serum gB antibody titers and serum level of total IgG (not shown).

TABLE IV. Immunologic Parameters and Median Antibody Titers of Treated and Untreated AIDS Patients With HCMV End-Organ Disease

Study group (no. patients)	Median CD4+[a]	Median IgG[b]	Antibody type (Median titer) Anti-gB	Neutralizing
Untreated (16)	13	1,973.0	80	80
Treated (9)	24	2,334.0	640	640

[a]CD4+ counts in cells/mm^3.
[b]Total IgG levels in mg/dl.

TABLE V. Anti-gB and Neutralizing Median Antibody Titers in Patients With HCMV Disease and Positive or Negative Antigenemia and Viremia Tests

Test result (no. of patients)	Antibody titer (Median/Range) Anti-gB	Neutralizing
Antigenemia		
Positive (15)	80/(40–640)	80/(40–160)
Negative (10)	640/(80–1,280)	480/(80–640)
Viremia		
Positive (20)	80/(40–640)	80/(40–640)
Negative (5)	640/(640–1,280)	640/(320–640)

As expected, HIV-infected persons (both HIV-1 asymptomatic and AIDS patients) had gB antibody titers significantly higher ($P = < 0.005$) than those of immunocompetent persons. Likewise AIDS patients without HCMV disease had significantly more anti-gB antibodies in sera than HIV-1 asymptomatic individuals ($P = 0.0051$).

TABLE VI. IgG Subclass Antibody Response to HCMV gB in Study Groups

Study group (no. patients)	IgG Subclass response to gB (no. reactive sera)/(median titer)[a]			
	IgG1	IgG2	IgG3	IgG4
AIDS with HCMV disease (25)	25/160	4/10	14/10	1/10
AIDS without HCMV disease (25)	25/160	2/10	13/10	1/10
HIV-1 infected asymptomatic (28)	28/80[b]	7/10	5/10	0/0
Immunocompetent controls (19)	19/80[c]	3/10	2/10	0/0

[a]Median titer of reactive sera.
[b]Sum of ranks:707.0.
[c]Sum of ranks:421.0.

Subclass Antibody Response to gB

gB-specific IgG subclass antibodies were then measured to determine whether either a deficient or an abnormal IgG subclass response could be observed in individuals with AIDS and HCMV end-organ disease in comparison with AIDS patients who remained free of HCMV-related disease. Data are summarized in Table VI. Anti-gB IgG1 antibodies were detected in all sera from both groups of patients and represented the main subclass antibody response to gB in both study groups. In fact, with minor discrepancies, anti-gB IgG1 antibody titers paralleled those of gB-specific total IgG antibodies. Median anti-gB IgG1 titers were similar in both groups of patients. Aproximately half of sera from both patient's groups had anti-gB IgG3 antibodies. Only a few sera contained anti-gB IgG2 and IgG4 antibodies, and these were distributed equally among both study groups. In addition, the gB-specific IgG subclass antibody respose of AIDS patients either with or without HCMV disease appeared to be qualitatively similar to that of HIV-1 infected asymptomatic individuals and immunocompetent subjects (Table VI).

Neutralizing Antibodies

In the light of the data on the quantitative response to gB, the complement independent neutralizing activity of sera was measured to determine whether identical differences existed between study groups. As expected, there was a close correlation between the neutralizing capacity of sera and anti-gB antibody titers ($r = 0.601$, 95% CI = 0.452–0.718, $P = < 0.0001$). As shown in Table II AIDS patients with HCMV-invasive disease had significantly fewer neutralizing antibodies than those without disease ($P = 0.0287$). Among the former patients, those who received specific anti-HCMV treatment had significantly more neutralizing antibodies than those who remained untreated ($P = 0.0025$) regardless of concurrent HCMV-associated disease (Table IV). In this context, untreated patients with retinitis alone displayed neutralizing antibody titers comparable to those found in untreated patients with other clinical conditions ($P = 0.795$). Globally, untreated patients had neutralizing antibody titers significantly lower than AIDS patients who remained free of HCMV end-organ disease ($P = 0.0001$). On the other hand, AIDS patients with HCMV disease with a positive antigenemia test had significantly fewer serum neutralizing antibodies than those with a negative test ($P = 0.0002$; Table V). Likewise, viremic patients had a lower median neutralizing titer than patients with a negative test ($P = 0.0016$). Finally, as expected on the basis of data on the quantitative response to gB, neutralizing activity of sera from AIDS patients and HIV-1 infected asymtomatic individuals was significantly higher than that of normal immunocompetent individuals (Table III), and patients with AIDS either with or without HCMV-associated disease had more neutralizing antibodies than HIV-1 asymptomatic subjects (Table III).

DISCUSSION

The aim of the present study was to determine whether HIV-1 infected patients who develop symptomatic HCMV-invasive disease display a deficient antibody response to HCMV when compared with matched patients free of HCMV disease. The data show that gB-specific and complement independent neutralizing antibodies, as well as antibodies to whole virus, are generated to higher levels in HIV-1 infected persons than in normal immunocompetent individuals. This is particularly noticeable in patients with AIDS and severely depressed CD4+ cell counts (+50 CD4 cells/mm^3) either with or without HCMV-associated disease who showed the highest antibody titers among our study groups. Differences in the anti-HCMV antibody levels between groups comprising HIV-1 infected individuals could not be attributed to differences in the mean serum IgG levels, since these were comparable in all three study groups. The results agree with those reported by others [Boppana et al.,1995; Lucht et al., 1993; Rasmussen et al., 1994] and indicate that in the presence of sufficient antigenic stimuli a quantitatively relevant humoral response to HCMV can be elicited despite the severe dysfunction of cellular immunity which is presumed to occur in the presence of extremely low CD4+ cell counts. It is uncertain, however, whether such antibody response is qualitatively similar to that of immunocompetent individuals given the presumably depressed capacity of the cellular immune system to process T-cell dependent antigens.

The group of patients with AIDS and HCMV end-organ disease had anti-whole virus and anti-gB antibody titers quite similar to those of the group of AIDS patients without HCMV disease, though the latter had a significantly higher mean neutralizing titer than the former. Nevertheless, the significance of such a com-

parison was questioned in view of the very distinct clinical profiles of the individuals belonging to this group. In this context, the recent history of anti-HCMV treatment had a major impact upon the levels of anti-gB and neutralizing antibodies recorded. Regardless of the HCMV-related disease present, patients who had received anti-HCMV specific treatment before sampling exhibited significantly higher levels of these antibodies than those who were not treated. Most treated patients did not have antigenemia and viremia, whereas the majority of untreated patients were antigenemic and viremic at the time of sampling. Considering the efficacy of systemic anti-HCMV therapy in reducing blood viral loads [Gerna et al., 1994], and the fact that positive antigenemia and viremia tests reflect the presence of a high systemic viral burden [Gerna et al., 1994; Zipeto et al., 1995], our data suggest strongly the existence of an inverse correlation between the systemic viral loads and the anti-gB and neutralizing antibody levels measurable by our assays. However, validation of this assumption requires a quantitative estimation of viral loads in addition to measuring antibody levels, which unfortunately could not be carried out this study. In support of this view, Shoppel et al. [1997] found an inverse correlation between antibody titers against the immunodominant epitope AD-1 located on gp55 (carboxy-terminal fragment of gB) and HCMV DNA positivity in blood cells in liver transplanted patients. Likewise, Rasmussen et al. [1995] reported on the failure to detect HCMV DNA in blood cells from HIV-1 infected patients who displayed very high anti-gB antibody levels. A plausible explanation for our finding is that when the systemic viral burden is high, a quantitatively relevant fraction of serum gB-specific and neutralizing antibodies are complexed with free virus or cell-associated viral proteins and therefore escape detection by immunoassays that only detect free antibody. In this context, the consumption of biologically active antibodies as a result of virus clearance [Krause et al., 1997] might predispose to the establishment or progression of HCMV disease. In this sense, anti-gB and neutralizing antibodies might not in themselves prevent the development of HCMV disease, yet could modulate severity and progression of the disease by reducing and keeping viral loads low. However, neither the possibility that a dramatic decrease in the level of measurable antibody preceded the increase in virus load nor the hypothesis that the occurrence of HCMV end-organ disease in our patients was directly related to a deficient antibody response to HCMV cannot be ruled out definitively by the data. To address this question we are monitoring periodically anti-gB and neutralizing antibody levels and plasma viral loads in a number of AIDS patients at high risk of developing HCMV-associated disease. Contrarily to the effect of specific therapy, the anatomic location and spread of HCMV disease present appeared to have only a minor impact upon the levels of anti-gB and neutralizing antibodies. In this sense, among patients who remained untreated, those with retinitis alone had comparable antibody titers to that of patients with any other clinical condition. Nevertheless, and given the limited number of patients with HCMV diseases other than retinitis who were subject to study, definitive conclusions require further study.

Biological functions of IgG antibodies are known to vary among different IgG subclasses [Spiegelberg, 1974]. Since abnormalities in B-cell maturation have been reported in AIDS patients [Lane et al., 1983], we examined the antibody subclass responses against gB in the patients to determine whether any specific defect could be detected. The anti-gB IgG subclass patterns in HIV-1 infected individuals were qualitatively similar to those found among immunocompetent individuals regardless of the stage of disease and the presence or absence of HCMV-associated disease in the former individuals. In agreement with earlier reports [Mathiesen et al., 1988, 1992; Sundqvist et al., 1986; Urban et al., 1994], the main IgG subclass response to gB in the patients was of types IgG1 and IgG3, as occurs in immunocompetent individuals with primary or past HCMV infections [Linde et al., 1983]. Thus, our study failed to show any relevant abnormality in the IgG subclass antibody response to gB in AIDS patients with symptomatic HCMV-associated disease in comparison to patients without HCMV disease.

Several studies have addressed the role of specific anti-HCMV antibodies in affording protection against HCMV disease in AIDS patients. Rasmussen et al. [1994] showed that anti-gB antibody titers did not significantly differ between patients with and without retinitis, thus concluding that antibodies of such specificity may not be helpful in preventing HCMV disease. These researchers, however, did not mention whether patients with retinitis had or had not received recently anti-HCMV drugs or whether patients were antigenemic or not at the moment of sampling; a strict comparison with our study therefore seems unfeasible. In a different study, Boppana et al. [1995] examined the anti-gB and HCMV neutralizing antibody responses in a population of AIDS patients with HCMV retinitis and found that patients with a slowly progressive disease had higher mean neutralizing antibody titers than rapid and intermediate progressors. Likewise, and although statistical significance was not reached, mean anti-gB antibody titers measured by ELISA appeared to be higher in the slow progressors when compared with intermediate and rapid progressors. Unfortunately, no reference to the virological features of patients was made in this study. These researchers concluded that HCMV neutralizing antibodies provide some protective activity for the host and delay progression of the disease. Our data support indirectly this assumption.

Additional in vivo studies are required to prove consistently that biologically active anti-HCMV antibodies provide some protective antiviral activity to either AIDS patients at high risk for developing HCMV disease or to those with established disease. If this were to be the case, then such a subset of patients would ben-

efit from the administration of exogenous antibody either alone or in combination with antiviral drugs. In this context, humanized antibodies to HCMV with selected specificities [Hamilton et al., 1997] and defined functional activities might be potential agents for the treatment, prevention or both of HCMV-associated diseases in AIDS patients. To this end, antibodies to HCMV-gB with potent neutralizing activity might constitute a feasible alternative in the near future.

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